

# **Interactions of Small-Scale Physical Mixing Processes with the Structure, Morphology and Bloom Dynamics and Optics of Non-Spheroid Phytoplankton**

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## **LONG-TERM GOALS**

Our long term goal is to understand the ecology of phytoplankton, especially the large, colonial diatoms which frequently dominate the flora of coastal shelves, upwelling areas, fjords and banks. We are interested in ways in which species-specific properties, including cell and colony size and shape interact with small-scale physical mixing processes to regulate the spatio-temporal distribution of diatoms. We wish to understand these processes in sufficient detail to be able to predict bloom dynamics, size structure and the impact of species-specific characteristics of the phytoplankton on ocean optics.

## **OBJECTIVES**

Our objectives are to document the spatio-temporal patterns of distribution of large, or “net” phytoplankton in the coastal ocean from a species-specific, and size/shape specific perspective, and to investigate the role that physical processes, operating at both the scale of populations and at the scale of individual cells and colonies play in controlling the patterns of distribution. In FY2001 we focused on four specific tasks:

(1) To conduct replicated laboratory experiments examining the effect of small-scale turbulence (6 treatments) on the colony size, morphology (shape) and growth rate of *Eucampia zodiacus*. This common coastal diatom was chosen because it was abundant in our 1998 field samples from East Sound, and because it forms helical colonies up to several millimeters in length, which are variable in morphology.

(2) To develop microscopic imaging techniques, and microscopic video-cinematography protocols for both phytoplankton and zooplankton for use in current laboratory experiments, future field work, web site development and video presentations.

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- (3) To evaluate the published phytoplankton, zooplankton and bioluminescence literature to determine whether studies in addition to those of the ONR-funded 'Thin Layers Experiments' provide evidence for the existence of thin layers in other areas of the world's coastal oceans.
- (4) To add a new section to the Critical Scales web site based on the literature review.

## APPROACH

We use both field and laboratory experiments to investigate the role that interactions between biological and physical processes play in regulating the distribution of phytoplankton in the coastal ocean. Field work is conducted in close collaboration with Donaghay's, Holliday's and Dekshenieks' ONR-funded programs. No field experiments were conducted during FY2001.

Laboratory: Laboratory experiments are important because they aid in the interpretation of species-specific observations made in the field. Previous experiments on several different kinds of chain forming diatoms demonstrated that when grown under non-turbulent conditions, colonies of up to cm-scale length were common. When exposed to sudden turbulence, as might be experienced during a storm, colonies fragmented. The resulting size-frequency spectrum was a function of both the level of turbulence, and the particular species. Our new experiments asked additional questions: when grown in a turbulent environment, do diatoms alter their structure/size/shape? We grew *Eucampia zodiacus* in 5 levels of small-scale turbulence (epsilon values from  $10^{-8}$  to  $10^{-3} \text{ m}^2 \text{ sec}^{-3}$ , quantified with a Sontec Acoustic Doppler Velocimeter), plus a quiescent control. We quantified growth rate (both as cell numbers, and as fluorescence measured with a WetLabs ECO-DFLS digital fluorometer), *in situ* particle length, and several morphological characters, including the tightness (pitch) of the helices, and the size of the connections between adjacent cells in a chain.

Literature review: Recognition of 'thin layers' as distinct phenomena is relatively recent. Documentation of thin layers of plankton, and elucidation of mechanisms contributing to their formation, maintenance and dissipation has depended on researchers asking the correct set of questions, and developing instrumentation capable of collecting data at the appropriate spatial and temporal scales to address those questions. Dedicated experiments have been carried out in only a few geographic locations. However, based on research to date we feel that thin layers are likely to be a widespread phenomenon. Since many older papers might contain evidence of thin layers, even if they weren't labeled as such, we conducted a literature review. Computerized library data bases were of little assistance. Instead, we worked backwards from references listed in recent phytoplankton, zooplankton and bioluminescence papers, and examined data/figures for layered structures.

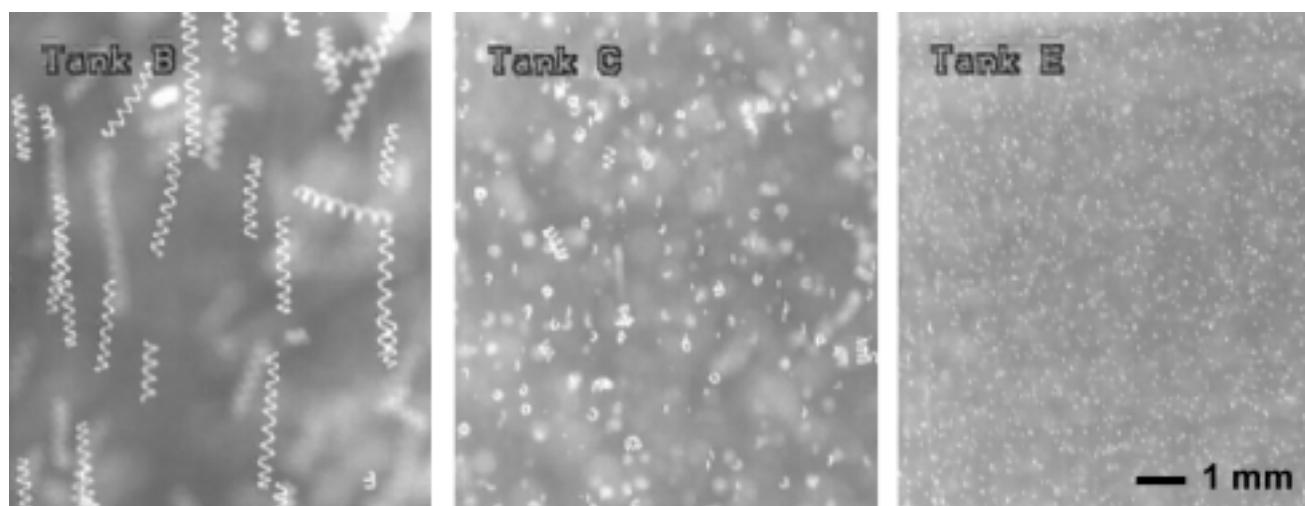
Imaging and Cinematography: Off-the-shelf equipment acquired under DURIP Award N00014-99-1-0598 has given us the capability to acquire high quality digital images and digital video using a variety of microscopes and macro-lenses. However, in order to effectively film plankton, it has been necessary to design and build additional custom parts and accessories. Development and enhancement of this system is a continual process.

## WORK COMPLETED

Laboratory: We completed 3 replicated sets of experiments on the diatom *Eucampia zodiacus*. The data has been analyzed, and we are currently preparing the results for publication. Our results clearly demonstrate that small-scale turbulence affects both colony size and morphology of *E. zodiacus*.

Particle, or colony length is directly related to the level of turbulence under which the diatom is grown (Figure 1). Helical colonies up to 4 mm in length, each composed of hundreds of cells, were formed at epsilon values  $\sim 10^{-8}$  to  $10^{-7} \text{ m}^2 \text{ sec}^{-3}$ . Only short fragments of colonies were formed at epsilon of  $\sim 10^{-6}$  to  $10^{-5} \text{ m}^2 \text{ sec}^{-3}$ . At epsilon  $\sim 10^{-4}$  to  $10^{-3} \text{ m}^2 \text{ sec}^{-3}$ , colonies were not formed at all: only single cells and pairs of cells occurred. Some turbulence appears to be necessary in order for *E. zodiacus* to form morphologically normal colonies. In the non-stirred control tank, many colonies were abnormally twisted. If formed, helices were often irregularly coiled.

We discovered that *Eucampia zodiacus* has the ability to alter its morphology in response to the turbulent conditions under which it is growing. Over the range of epsilon values where colonies were formed ( $\sim 10^{-8}$  to  $10^{-6} \text{ m}^2 \text{ sec}^{-3}$ ), the pitch of the helix (i.e. tightness of coiling) decreased with increasing turbulence. Differing levels of turbulence lead *E. zodiacus* to alter the mechanical strength of the connection between cells. The silicon processes that connect adjacent cells in a colony were largest under the conditions that led to formation of the longest colonies.



**Figure 1. In situ video stills of *Eucampia zodiacus* growing under continuous turbulence in 20 liter laboratory tanks. Three of six treatments are shown. Tank B: epsilon  $\sim 4 \times 10^{-7} \text{ m}^2 \text{ sec}^{-3}$ , Tank C: epsilon  $\sim 3 \times 10^{-6} \text{ m}^2 \text{ sec}^{-3}$ , Tank E: epsilon  $\sim 3 \times 10^{-4} \text{ m}^2 \text{ sec}^{-3}$ . The scale is the same for all photos. [Photos: Helical colonies several millimeters in length are formed at low levels of turbulence. At higher levels, colonies are not formed, and only single cells or pairs of cells occur.]**

Growth rates calculated from cell counts, and from fluorescence measurements were similar. It appeared that higher growth rates were achieved at low (epsilon  $\sim 10^{-8}$  to  $10^{-7} \text{ m}^2 \text{ sec}^{-3}$ ) levels of turbulence, however this result was not statistically significant. The final yield was similar in all treatments: the photographs in Figure 1 depict approximately the same concentration of cells in each tank.

Literature review: Approximately 50 published papers were identified that provided evidence of thin layers. Of these, about 35 included clear examples. Most were located in US and northern European coastal waters, and the Mediterranean. This is not surprising, since these regions are near major oceanographic institutions and have been the sites of intense oceanographic research. We have been

unable to identify examples of thin layers in the southern hemisphere, presumably because southern waters have been less thoroughly studied than the North Atlantic and North Pacific. For each paper, we entered the data that was available into a master spreadsheet. Targeted information included location of the study, basin type, date, number of layers in profile, as well as layer thickness, depth, horizontal extent, intensity, duration, and organism type. We also recorded profiling/sampling methods, physical and chemical data, +/- toxins, +/- bioluminescence, and any controlling mechanisms proposed by the authors. Selected information has been entered into an ArcInfo-based GIS system for potential future integration with our own data. In order to summarize this information in a way that is easy to access, we are adding a major section to the Critical Scales web site. It includes an interactive world map locating sites where thin layers have been reported, a list of references, and a page for each paper. Each summary page includes/will include the paper's full citation, pertinent graphs, a location map constructed from World Vector Shoreline Plus data, illustrations of the organisms in the layers, and commentary. At present the web site is not publicly available, but if you would like to preview it as a work-in-progress, email me (jrines@gso.uri.edu), and I will provide you with a temporary URL.

Imaging and Cinematography: Digital imaging and analysis equipment greatly facilitated the collection and analysis of experimental data on *Eucampia*. Using micro-cinematography, I have filmed approximately 50 kinds of motile plankton. Digital clips are being used to illustrate the Thin Layers Review web site, for educational presentations, and for web-based educational efforts (e.g. Jan Rines' Plankton Theater).

## RESULTS

Biological, physical and chemical processes all influence the distribution and population dynamics of phytoplankton. The critical issue is to identify the temporal and spatial scales at which different processes are important, or primarily responsible for producing the patterns and characteristics of interest. Under past and current ONR funding, we have clearly demonstrated that physical processes influence phytoplankton at the scale of populations, and at the scale of individual particles (organisms).

Populations: In East Sound, WA, we have identified four general patterns in the species-specific, vertical distribution of phytoplankton. Three of these included thin layers, either in the upper water column, or near the bottom. In the fourth, phytoplankton were well mixed in the water column above the pycnocline. Since extensive physical data was collected by Donaghay, Holliday, Dekshenieks and Osborn, we were able to demonstrate that advective physical processes operating on a regional scale in the southern part of the Strait of Georgia were responsible for producing three of the species-specific, vertical patterns in our data. The fourth was most likely the result of aggregation and settling of a surface phytoplankton bloom (Alldredge et al., accepted). Our literature review demonstrated that thin layers have been found in many areas in addition to East Sound. Although some reports were from fjords, or fjord-like environments similar to East Sound, layers were also reported from estuaries, basins, plumes, and a variety of coastal waters and seas. In addition to layers of phytoplankton, layers of aggregates, zooplankton and bacteria have been reported. Layers may contain high concentrations of toxic, or otherwise harmful algae, and/or they may be bioluminescent. They have rarely been studied in sufficient detail to document their spatial or temporal extent. Most biological studies did not contain sufficient physical and/or temporal data to determine the mechanisms controlling layer dynamics. Therefore, it is difficult to make direct comparisons between reports, or to draw generalized conclusions about oceanographic processes involved in thin layer formation, maintenance and dissipation. Further interdisciplinary studies are needed, in which biological data is overlaid on 4-dimensional physical data.

Individuals: Observations on live diatom colonies collected in East Sound suggested that small-scale turbulence, operating at the scale of individual particles is also important to understanding phytoplankton dynamics. In the lab, we have learned that both sudden, and continuous turbulence can regulate the size-frequency spectrum of particles. We are especially excited by our recent discovery that the diatom *Eucampia zodiacus* can adapt to variations in turbulence by altering its size and morphology – in fact, some turbulence appears necessary for normal colony formation. Several of the morphological variations we observed in the field were reproducible in the lab when the diatom was grown under different levels of turbulence, suggesting that it may be possible to use field morphology as an indicator of turbulent conditions over the time scale at which individual colonies grow.

We are now working on three new manuscripts on the importance of small-scale physical processes to phytoplankton dynamics, to be submitted for publication.

## **IMPACT/APPLICATIONS**

Demonstration of the impact of physical processes on phytoplankton distribution, particle size and shape has applications in the study of marine planktonic food chains, and for ocean optics.

## **RELATED PROJECTS**

This project benefits from collaboration with Donaghay, Holliday, Dekshenieks & Osborn. Phytoplankton field data from East Sound has been provided to Alldredge, and has been incorporated into a manuscript on which she is first author (below). Information was also provided to Twardowski & Donaghay (2001).

In 1999 Rines received DURIP funding (N000149910598) to enhance microscopy, digital imaging and image analysis capabilities. In addition to benefiting our primary ONR research, this equipment has been used to create digital video used in the following projects and presentations:

Jan Rines' Plankton Theater (<http://thalassa.gso.uri.edu/plankton/theater>)

Jan Rines: Exploring the Dazzling Diversity of Microscopic Life in the Sea. presentations at the Naval War College, Newport RI, University of New Hampshire and New Hampshire Sea Grant, Roger Williams University, and upcoming at Maine Sea Grant.

Jennifer Specker: Why are Flounder Flat? Naval War College, Newport RI, August 2001.

Graduate School of Oceanography, URI: Presentation to Admiral West, Oceanographer of the Navy.

Photomicrographs of phytoplankton in ONR exhibit (2000) – Office of Naval Research Management Information Center, Fran Rothwell Reception Room.

## **REFERENCES**

Twardowski, M.S. & P.L. Donaghay (2001) – Separating in situ and terrigenous sources of absorption by dissolved materials in coastal waters. *Journal of Geophysical Research* 106: 2545-2560.

## PUBLICATIONS

Aldredge, A.L., T.J. Cowles, S. MacIntyre, **J.E.B. Rines** P.L. Donaghay, C.F. Greenlaw, D.V. Holliday, M.M. Dekshenieks, J.M. Sullivan & R. Zaneveld (accepted) - Occurrence and mechanism of formation of a dramatic thin layer of marine snow in a shallow Pacific fjord.

Dekshenieks, M.M., P.L. Donaghay, J.M. Sullivan, **J.E.B. Rines**, T.R. Osborn and M.S. Twardowski. (in press) – Temporal and Spatial Occurrence of thin phytoplankton layers in relation to physical processes. Marine Ecology Progress Series.

**Rines, J.E.B.**, P.L. Donaghay, M.M. Dekshenieks, J.M. Sullivan and M.S. Twardowski (in press) – Thin Layers and Camouflage: Hidden *Pseudo-nitzschia* populations in a fjord in the San Juan Islands, Washington, USA. Marine Ecology Progress Series.